CLAIMS

1. A method for producing a capsular polysaccharide from an encapsulated bacterium comprising:

- culturing the encapsulated bacterium in a suitable culture medium at a suitable pH and temperature, while adjusting the pH of the culture medium to a constant value with a base or acid until adjustment with respectively base or acid is not possible anymore
- terminating the culturing just before the increase or decrease of the pH starts to slow down , preferably by cooling to below the temperature used for culturing
- harvesting the fermentation broth
- optionally, recovering the polysaccharide from the culture medium.

2. Method according to claim 1 wherein the fermentation is terminated within about 6-14 hours after the start of the fermentation.

3. Method according to claim 1 or 2 wherein lysis is delayed by cooling to below 30°C, preferably below 25 or 20°C.

4. Method according to claim 3 wherein the pH of the culture medium is adjusted with base to a constant value of between 6.5 and 7.5.

5. Method according to claims 1-4 wherein the culture medium is used to culture a strain of *Haemophilus influenzae* type b.

6. Method for recovering a polysaccharide from a fermentation broth comprising:

- omitting the use of phenol, high-speed centrifugation, ultracentrifugation and chromatography,;
- maximally 4 precipitation steps.

7. Method according to claim 6 wherein the recovery includes:

- mixing the polysaccharide fraction with a cationic detergent
- adding alcohol until a concentration which is below the concentration necessary

for precipitating the polysaccharide.

8. Method according to claim 6 or 7 comprising:
- using a cationic detergent to precipitate the polysaccharide or part of the contaminants from the supernatant to obtain a first polysaccharide fraction;
- using alcohol to precipitate the polysaccharide from the first polysaccharide fraction to obtain a second polysaccharide fraction;
- subjecting the second polysaccharide fraction to an alcohol precipitation in the presence of an anionic detergent, whereby the alcohol is present in a concentration which is below the concentration at which the polysaccharide precipitates;
- precipitating the polysaccharide from the soluble fraction using alcohol to obtain a polysaccharide precipitate;
- dissolving the polysaccharide precipitate and subjecting it to concentration and diafiltration.

9. Method according to claim 8 wherein the polysaccharide is a capsular polysaccharide which has been produced according to the method of claim 1-5.

10. Method for producing a polysaccharide conjugate vaccine which method comprises:
- producing a polysaccharide according to the method of claims 1-5
- recovering the polysaccharide from the culture medium
- optionally, activating the recovered polysaccharide for conjugation
- conjugating the recovered polysaccharide to a protein carrier, preferably a toxoid
- optionally, purifying the polysaccharide-protein conjugate.

11. Method according to claim 10 wherein the polysaccharide is recovered from the culture medium by using a process according to claim 6 or 7.

12. Method according to claim 10 or 11 wherein the polysaccharide is subjected to controlled alkaline degradation in the presence of a bicarbonate/ carbonate buffer under

vigorous agitation before activation or conjugation.

13. Method according to claim 11 or 12 wherein the polysaccharide is activated and then purified by using a tangential flow filtration system.

14. Method according to claims 10-13 wherein the activated polysaccharide is conjugated to protein at a pH in the range of pH 4.0 to 6.5, wherein the pH is regulated by a buffer devoid of carboxylic acid groups.

15. Method according to claim 14 wherein the pH is regulated by a 2-morpholino ethanesulfonic acid (MES) buffer at pH 5.5 to 6.1.

16. Method according to claims 1-15 wherein the polysaccharide is polyribosyl ribitol phosphate.

17. Pharmaceutical composition comprising a polysaccharide or polysaccharide conjugate which is produced according to the method of claims 1-16.